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14. ABSTRACT (around 200 words) Perfluorocarbon emulsions (PFCs) can treat traumatic injuries (traumatic brain injury (TBI), hemorrhagic shock and burns) by enhanced delivery of oxygen. A class-based side effect of PFC (day 2-5 after infusion in 30-50%) may be thrombocytopenia (TCYP). The mechanism is inadequately investigated but is caused by reduced production or enhanced clearance (partial activation) of platelets (Plts). The United States Food and Drug Administration (FDA) requests investigation of the phenomenon to exclude Plt inflammatory/embolic safety risks. In phase one of the study, healthy juvenile sheep were used with a top load intravenous infusion of either PFC (Oxygent, n=7), Hespan (n=6), or naïve/saline control (n=6). Venous blood was sampled before the treatment (baseline) and at 0 minute, 3 and 24 hours, 4 and 7 days after treatment. Blood samples were measured for coagulation parameters including platelet count, fibrinogen, thrombin, CD62p etc. The results showed that the sheep's platelet count and fibrinogen level were not significantly reduced after PFC top-loaded for the 7 survival days. Platelet activation assay (CD62p) also showed no increase compared with control groups (naïve & Hespan). Morphologically, platelet activation was not significantly increased compared with baseline or controls. Therefore, treatment with Oxygent in healthy sheep did not cause massive or severe coagulopathy.					
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Introduction

Perfluorocarbon emulsions (PFCs) are a small volume robust (temperature stable, long storage life, portable) intravenous (i.v.) fluid, easily carried by medics/corpsmen to site of first contact. PFCs enhance O₂ solubility/diffusion from circulating red cells. PFCs have shown efficacy in animal models of hemorrhagic shock, tissue ischemia, decompression sickness (DCS), traumatic brain injury (TBI) and other important military applications. Our work and that of others demonstrated that PFCs enhance O₂ delivery at normal FiO₂ and that perhaps the most important aspect of PFC infusion was an enhanced O₂ delivery from native erythrocytes to tissues. Furthermore, it appears that PFCs enhance O₂ diffusion, thereby decreasing the barrier to non-polar gas movement made up of aqueous materials (plasma and extracellular fluids). However, a class-based side effect of PFC (day 2-5 after infusion in 30-50%) is thrombocytopenia (TCYP). The mechanism is inadequately investigated but is caused by reduced production or enhanced clearance (partial activation) of platelets (Plts). These safety concerns posed by the United States Food and Drug Administration (FDA) have to do with a potential risk of hemorrhage/thrombosis and inflammation related to PFC infusion. Casualty care for hemorrhage, gas embolism (blast and DCS) and TBI all involve degrees of inflammatory up-regulation and variable elements of coagulopathy. The current approved work is to answer safety and mechanism questions regarding causes/extent of thrombocytopenia after PFC infusion. Pertinent large animal models of normal and casualty scenarios will be investigated, thereby demonstrating whether the use of PFC in hemorrhage and blast TBI possess any added coagulopathic risk to future victims, compared to normal. Large animal models will examine specific causal hypotheses for TCYP and whether this exists as a class effect. In the end, the work will provide answers to questions blocking further development of PFCs. In this proposed study, the side effects of two PFC's on platelet count, structure and function will be tested. PHER-O₂ contains perfluorodecalin (88%), purified water and an emulsifier that allows the product to be administered intravenously. Perfluorodecalin is a biologically inert substance that is not metabolized by the body but rather is excreted from the body through normal respiration. Oxygent, another resuscitation product, contains perflubron emulsion (60%, w/v) and has a similar O₂ carrying capacity like PHER-O₂. In the present study, the specific aims are to answer the following: #1 Whether PFC infusion activates Plts in vivo, #2 Whether Plt/white cells clumps (microaggregates) occur, and #3 Evaluate the mechanisms of partial Plt activation (if it occurs).

Body of Report

Material and Methods: All animals received humane care in compliance with the "Eighth Guide for Care and Use of Laboratory Animals", prepared by the National Academy of Sciences and published by the National Institutes of Health. This study was approved by the Association for Assessment and Accreditation of Laboratory Animal Care (AALAC) certified Virginia Commonwealth University Institutional Animal Care and Use Committee (IACUC) and was also approved by the USAMRMC Animal Care and Use Review Office (ACURO).

Study Design: A randomized ovine (20-30 kg) normal animal model was used. Animals were anesthetized, instrumented and had either hetastarch or an equal volume of one of three commercially available PFCs administered. At key time points blood samples were tested for Plt/white cell activation and morphology (Plt number, Plt white cell aggregates, flow cytometry-glycoprotein expression) as well as other coagulation data (RoTEM, Platelet Shear Modulus, PFA-100 and Plt aggregometry) and complement expression. Samples were examined with scanning electron microscopy for Plt and white cell transmission. In years 2-3 the ovine studies will turn to a polytrauma model of combined hemorrhagic shock and blast TBI. Volume resuscitation will occur with either hetastarch or PFC. Similar

studies of Plt and white cell activation will be carried out.

Subjects: When Juvenile sheep (Dorset/Dorper cross, 25-30 kg) were shipped to VCU DAR facility, general health checkup was taken immediately by a veterinarian, including measurements of sheep body temperature, heart rate and respiratory auscultation. Venous blood sample were drawn for complete blood count (CBC). Stool samples were examined for any parasite infections. Sheep were acclimated for 7 days in order to recover from shipping fever or to treat any potential infection. Sheep were randomized into one of three groups: PFC (Oxygent) group; Hetastarch (Hespan) group and Naïve or saline control group. In 2013, 26 sheep were shipped to VCU DAR facility. 3 sheep were used for top-load or hemorrhagic shock model development. 4 sheep were sick and were excluded from the study based on veterinarian advice. 19 animals were used for the study as follows: Oxygent group, n=7; Hespan group, n=6 and control group, n=6.

Animal procedures: Coagulopathy in sheep top loaded with PFC or hetastarch was assessed at baseline prior to compound administration and at time zero, 3 hours, 1 day, 4 days, and 7 days following infusion. Baseline venous samples via external jugular vein puncture were taken two days before top-load experiments. On procedure day, sheep were anesthetized with 4~5% isoflurane via vaporizer cart. Once unconscious, anesthesia was maintained with 2~3% isoflurane based on the anesthesia level assessment. Animals were transported to laboratory. Then, the animals were intubated and ventilated with mixed 70% nitrogen : 30% oxygen. The animal's neck area was shaved and disinfected with 70% ethanol and betadine as well as covered with a surgical drape. Local lidocaine was used to reduce pain. A jugular needle catheter (20 Gauge, 2 inch in length) was placed for PFC or Hespan infusion (3g/kg) over 15 minutes. Immediately following infusion, time zero blood sample was collected. The jugular catheter was removed and the puncture site sanitized. The initial top-load procedure was about 20~40 minutes. There was no dehydration during this short period. During the procedure, body temperature was maintained with pre-warmed heating blanket. Animal's heart rate and oxygen saturation were monitored. The animal was then transported back to DAR vivarium for recovery from anesthesia. Animals were monitored and weighed on a daily basis to ensure proper food intake and hydration. Note that animal venous blood was sampled via jugular vein puncture for baseline, 3 hours after top-load, 24 hours, 4 days and 7 days post top-load without anesthesia. Blood sampling without anesthesia is a common veterinary practice and minimizes respiratory distress and the potential for decreased food intake and dehydration from repetitive daily exposure to gas anesthesia.

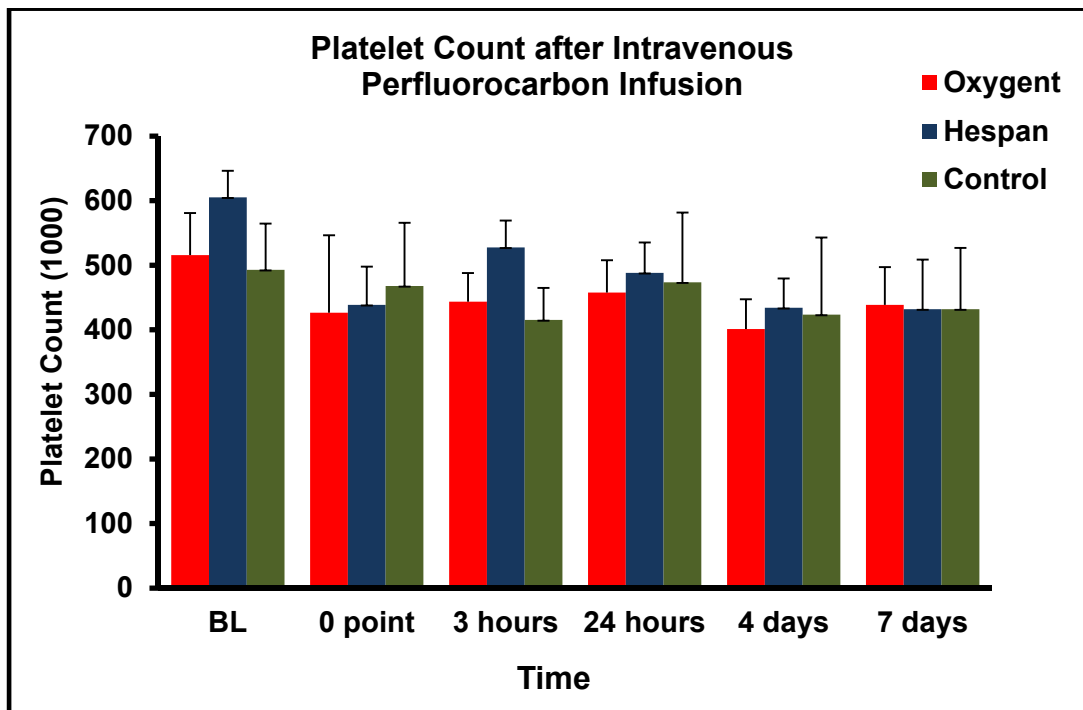
Study endpoints: **1. Sheep behavioral monitoring** was entirely observational and the sheep were in their own enclosure with the rest of their flock during the period of observation. Video cameras were used to monitor the sheep 24/7 before and after experiments. Scoring of the video records was done by an observer who was blind to the treatment status of the sheep in question and was scored based on the proportion of each day that the sheep spends actively moving around the enclosure, feeding, or lying down and inactive. After the conclusion of the experiment all animals were humanely euthanized. Sheep were video monitored from 2 days before top load through 7 days after the experiment. Screen monitoring and video record materials are protected and accessed only by authorized personnel following IACUC guide lines. **2. Blood sample analyses** included coagulopathy tests (see attached assay protocol); platelet count, blood biochemistry and platelet morphologic observation using scanning electron microscopy.

Statistical analysis: Power analysis based on sheep platelet mean number was used to estimate

animal numbers per experimental group. JMP pro 10.0 statistical software was used to analyze all blood sample results. Data distribution and one-way analysis of variance (ANOVA) were used to compare means. Data was compared among groups and within the group at different time points. Significant difference between means was p value less than 0.05 ($p < 0.05$).

Results (blood sample analyses)

- 1. Platelet count** Normal sheep platelet mean value was about 400,000 (range from 100,000 to 800,000). Our study data showed that platelet count did not change significantly when compared with control and Hespan groups. Oxygen group, $n=7$; Hespan group, $n=6$; Naïve/saline group, $n=6$



Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Groups	2	70938.13	35469.1	2.0034	0.1673
Error	16	283271.65	17704.5		
C. Total	18	354209.78			

Means for One-way Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
Control	6	493.027	54.321	377.87	608.18
Hespan	6	605.305	54.321	490.15	720.46
Oxygent	7	462.614	50.291	356.00	569.23

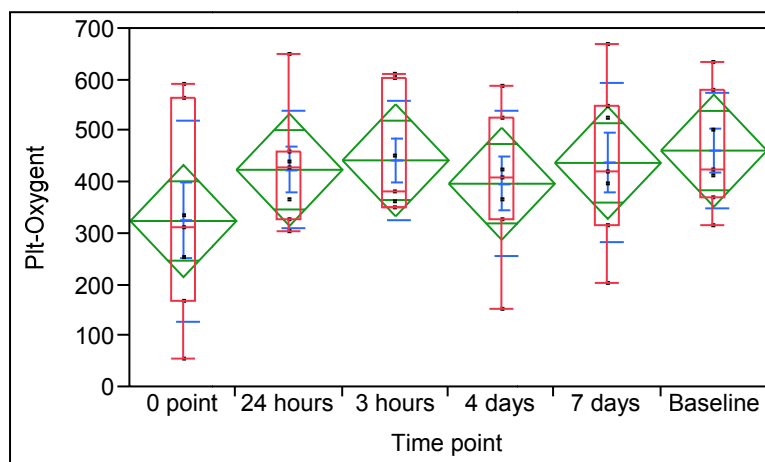
Std Error used a pooled estimate of error variance

Comparing platelet count at different time points within the group, there was no significant change among time points.

Fit Y by X Group

One-way Analysis of Plt-Oxygen By Time point

Means for One-way Anova of Oxygen group



Level	Number	Mean	Std Error	Lower 95%	Upper 95%
0 point	7	325.083	54.009	215.55	434.62
24 hours	7	425.084	54.009	315.55	534.62
3 hours	7	443.661	54.009	334.13	553.20
4 days	7	397.929	54.009	288.39	507.46
7 days	7	438.691	54.009	329.16	548.23
Baseline	7	462.614	54.009	353.08	572.15

Std Error used a pooled estimate of error variance

2. Platelet activation

morphological observation by Scanning Electron Microscopy

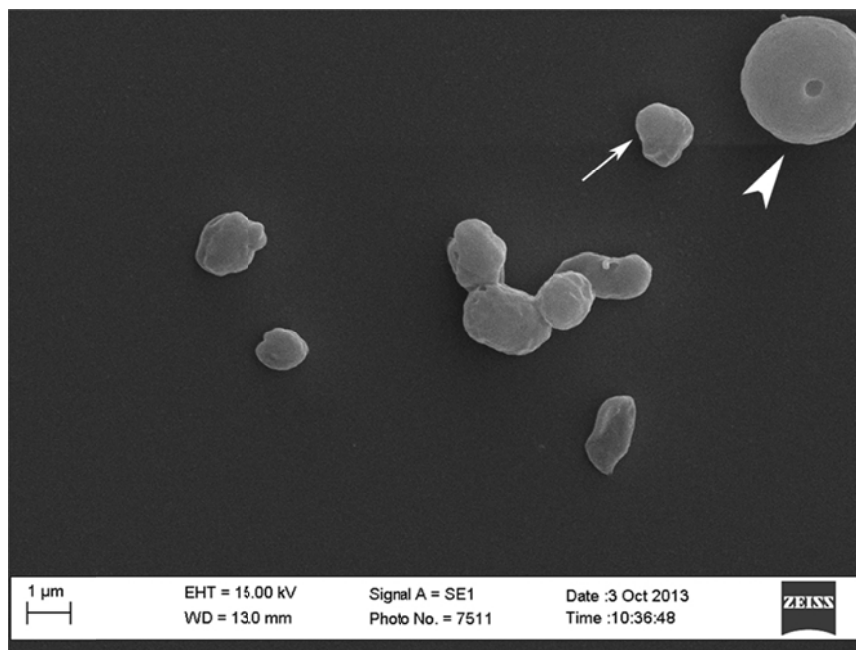


Figure1. Non-active platelets (white arrow) and red blood cells (arrow head). Non-active platelets were small in size with a smooth surface (measure bar = 1 μ m).

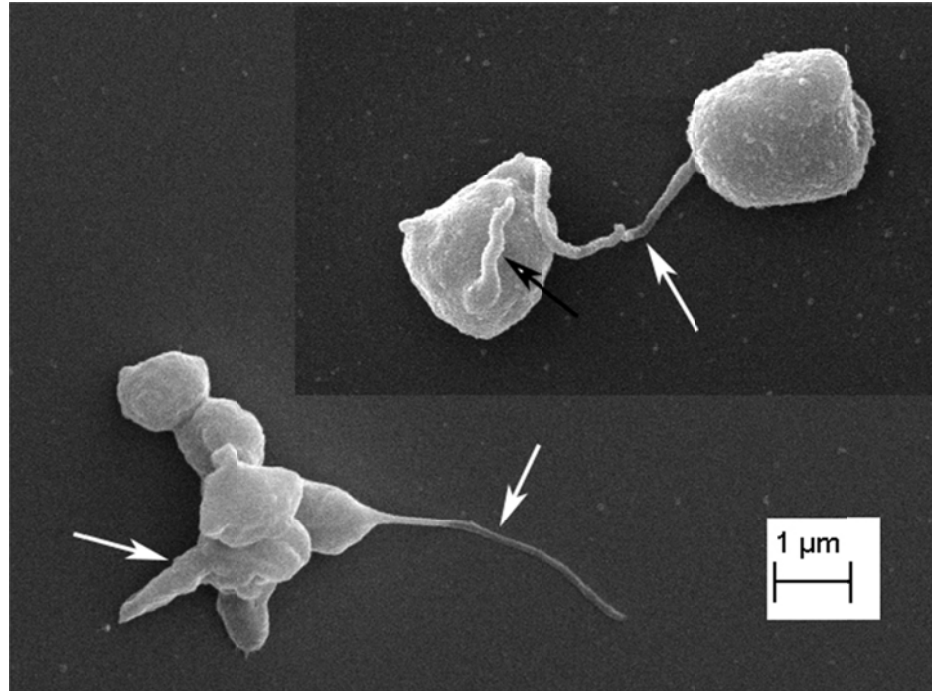


Figure2. Semi-active platelets had one or 2 pseudopods (white or black arrows) and increased surface size.

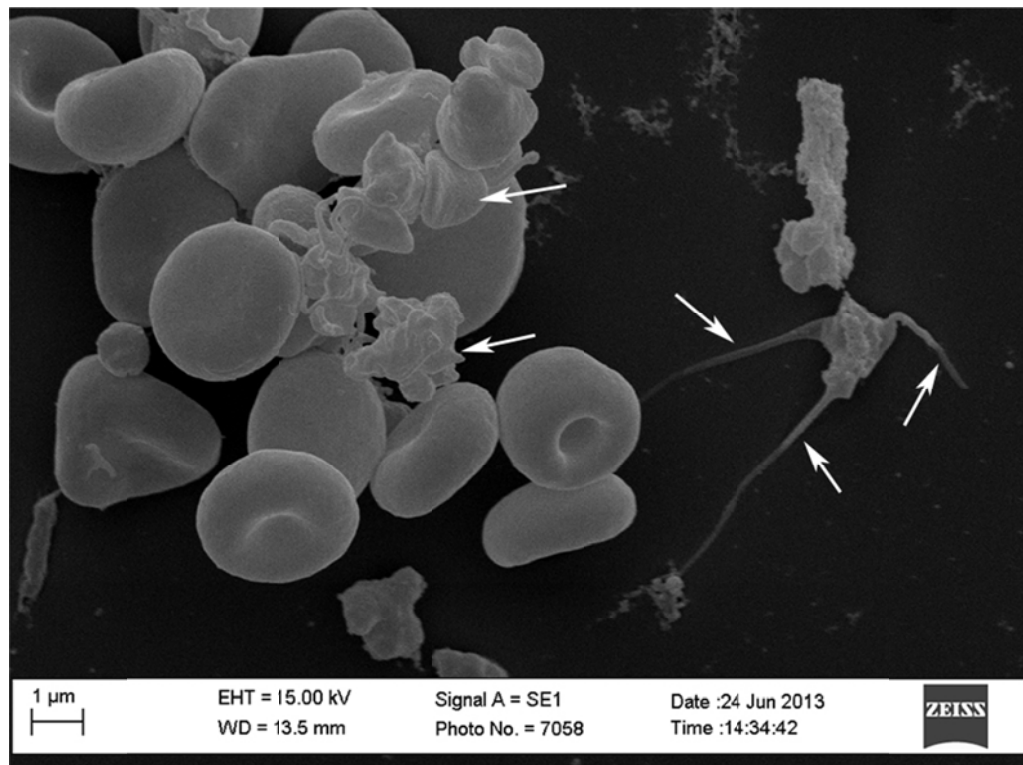


Figure3. Active platelets had 3 or more pseudopods (white arrows) and a rough or granular surface.

quantitative assessment of platelet activation

Criteria Used to Analyze Platelet Count Images

1. Counted total number of platelets on image (each single n value averaged 4~6 images)
2. Semi-activated platelet: there was 1 or 2 pseudopods with smooth surface
3. Full-activated: 3 or more pseudopods with rough surface.
4. Conjugated Platelets: platelets that had pseudopods connected each other as full-activated.

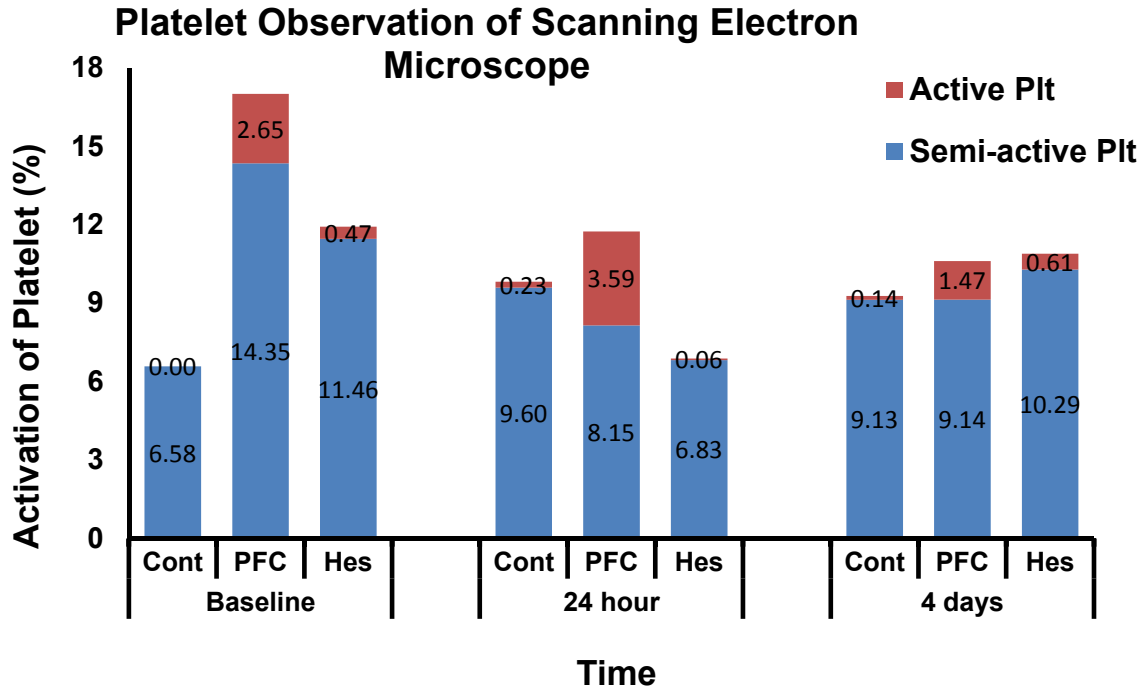


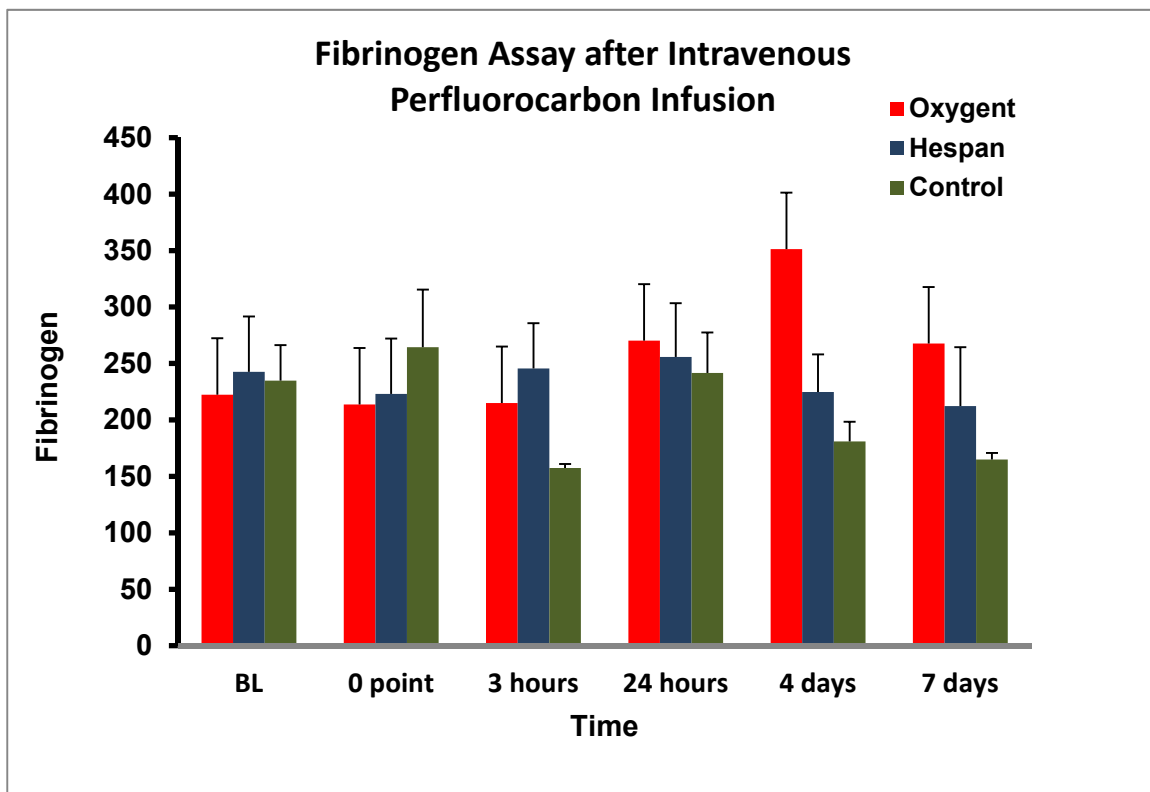
Figure4. Percentage of total platelets that were semi-active or active platelets. Cont = control group (n=6); PFC = Oxygent group (n=7); Hes = Hespan group (n=6). Plt = platelet. Data in Figure 4 calculated based on platelet counts described in the table below.

		Baseline			24 hrs			4 days		
		semi-active	active	Total plt count	semi-active	active	Total plt count	semi-active	active	Total plt count
Control n=6	Plt count	58.00	0.00	881.00	173.00	4.11	1803.00	60.00	0.95	657.00
	%	6.58	0.00	93.42	9.60	0.23	90.18	9.13	0.14	90.72
Oxygent n=7	Plt count	92.00	17.00	641.00	109.00	<u>48.00*</u>	1337.00	87.00	14.00	952.00
	%	14.35	2.65	83.00	8.15	3.59	88.26	9.14	1.47	89.39
Hespan n=6	Plt count	98.00	4.00	855.00	120.00	1.00	1758.00	169.00	10.00	1643.00
	%	11.46	0.47	88.07	6.83	0.06	93.12	10.29	0.61	89.11

* There was one case with a highly active platelet count. Red number indicates the total count of platelets.

Comparing the percentage of active platelets among groups and baseline, there were no significant changes in the number of active or semi-active platelets ($p > 0.05$).

3. Fibrinogen measurement



Fibrinogen measurement: There was no significant difference when the groups were compared after top-load with Oxygent or Hespan. Also, no significant difference was found within the groups when different time points were compared ($p > 0.05$). Even at day 4, Oxygent group showed higher fibrinogen measurement, but there was statistically no significant difference. (see following results)

Analysis of Variance at Day 4 post-top load.

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Groups	2	111555.49	55777.7	1.7699	0.2003
Error	17	535740.71	31514.2		
C. Total	19	647296.20			

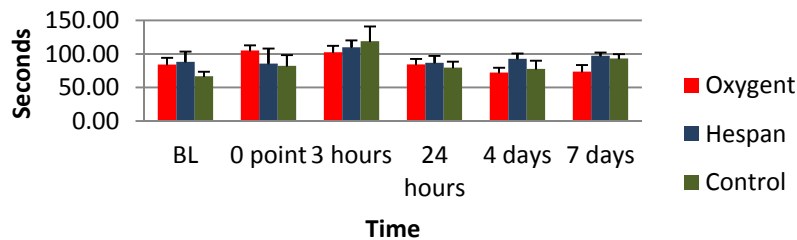
Means for One-way Anova at day 4

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
Control	6	181.000	72.473	28.09	333.91
Hespan	6	224.833	72.473	71.93	377.74
Oxygent	8	351.375	62.764	218.96	483.79

Std Error used a pooled estimate of error variance

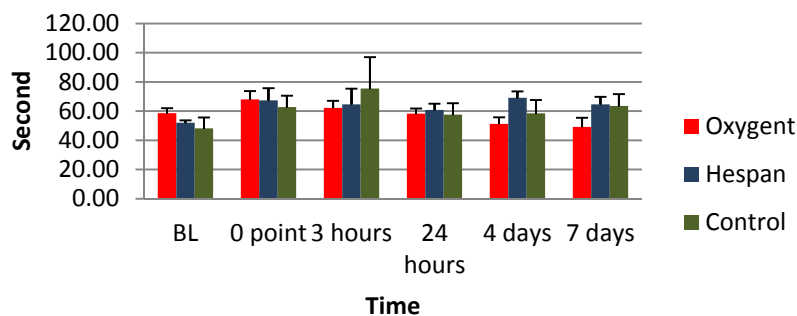
4. Clot formation time (Extem, Intem, Natem)

Clot Formation Time (EXTEM) after Intravenous Perfluorocarbon Infusion



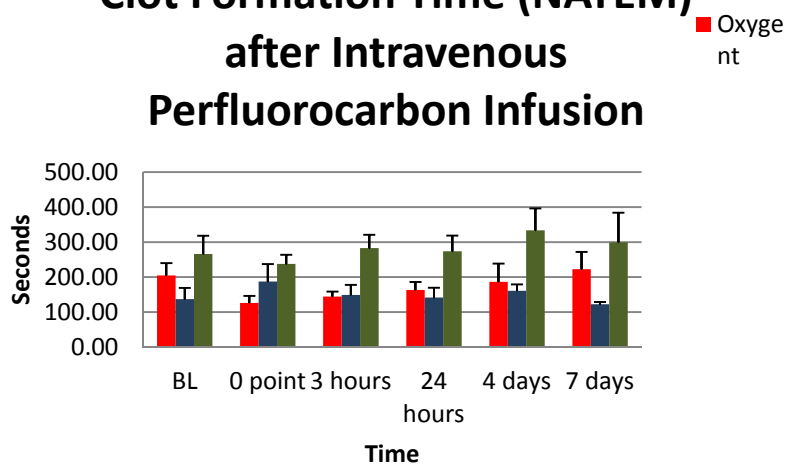
4a Clot formation time (Extem) measurement: There was no significant difference when groups were compared after top-load with Oxygent or Hespan. Also, no significant difference was found within the groups when different time points were compared ($p>0.05$).

Clot Formation Time (INTEM) after Intravenous Perfluorocarbon Infusion



4b Clot formation time (Intem) measurement: There was no significant difference when groups were compared after top-load with Oxygent or Hespan. Also, no significant difference was found within the groups when different time points were compared ($p>0.05$).

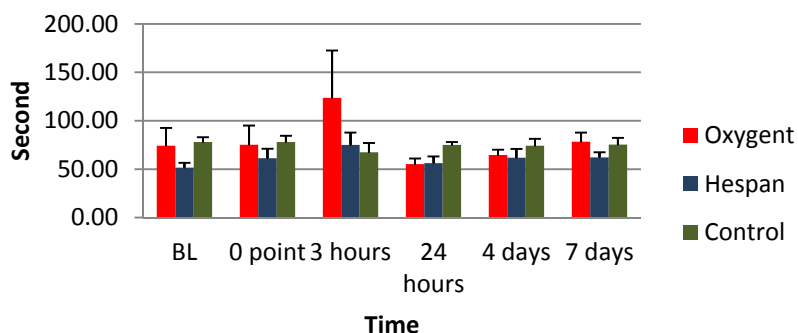
Clot Formation Time (NATEM) after Intravenous Perfluorocarbon Infusion



4c Clot formation time (Natem) measurement: There was no significant difference when groups were compared after top-load with Oxygent or Hespan. Also, no significant difference was found within the groups when different time points were compared ($p>0.05$).

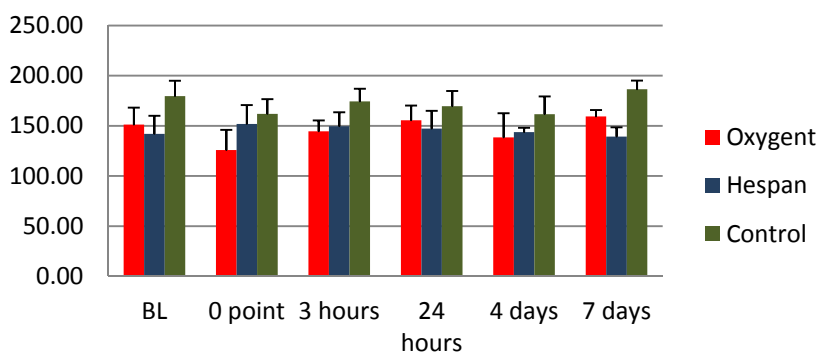
5. Clotting time (Extem, Intem, Natem)

Clotting Time (EXTEM) after Intravenous Perfluorocarbon Infusion



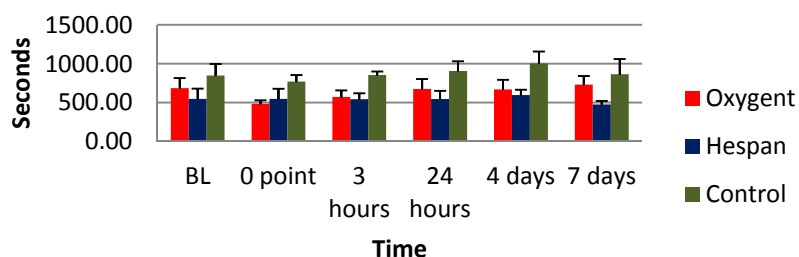
5a Clotting time Extem measurement: There was no significant difference when groups were compared after top-load with Oxygent or Hespan. Also, no significant difference was found within the groups when different time points were compared ($p>0.05$).

Clotting Time (INTEM) after Intravenous Perfluorocarbon Infusion



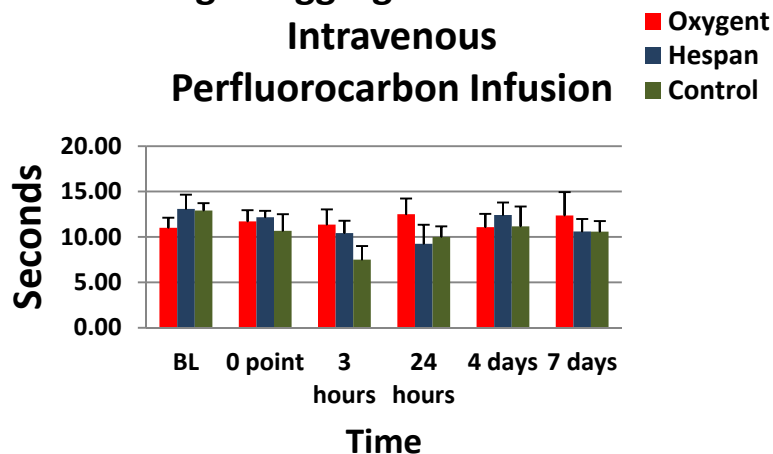
5b Clotting time Intem measurement: There was no significant difference when groups were compared after top-load with Oxygent or Hespan. Also, no significant difference was found within the groups when different time points were compared ($p>0.05$).

Clotting Time (NATEM) after Intravenous Perfluorocarbon Infusion



5c Clotting time Natem measurement: There was no significant difference when groups were compared after top-load with Oxygent or Hespan. Also, no significant difference was found within the groups when different time points were compared ($p>0.05$).

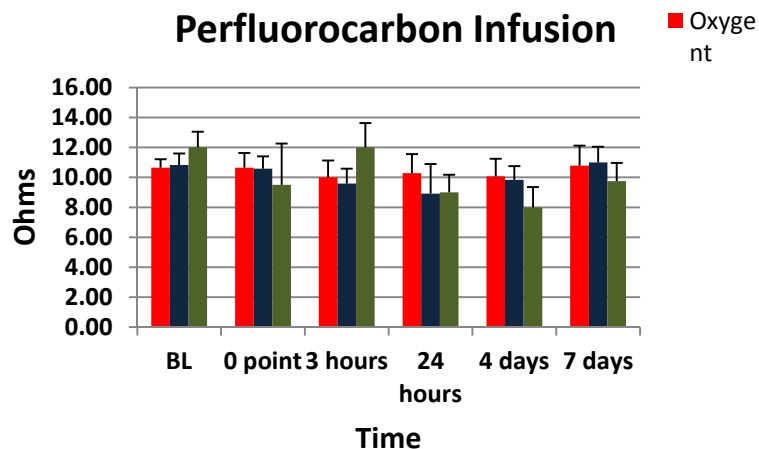
Collagen Aggregation Time after Intravenous Perfluorocarbon Infusion



6. Collagen Aggregation

There was no significant difference when groups were compared after top-load with Oxygent or Hespan. Also, no significant difference was found within the groups when different time points were compared ($p>0.05$).

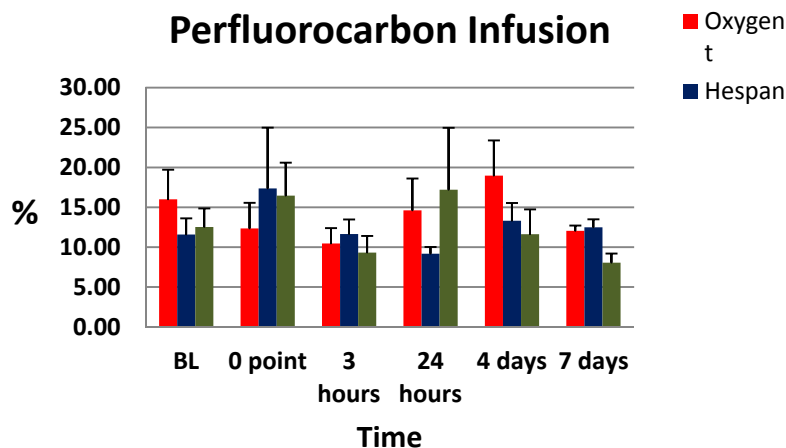
ADP Aggregations after Intravenous Perfluorocarbon Infusion



7. ADP Aggregation

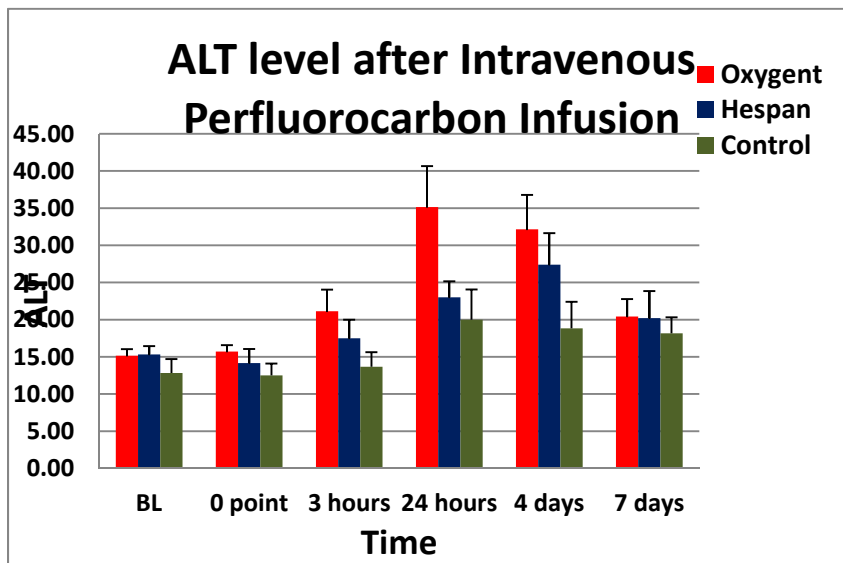
There was no significant difference when groups were compared after top-load with Oxygent or Hespan. Also, no significant difference was found within the groups when different time points were compared ($p>0.05$).

Platelet CD62p after Intravenous Perfluorocarbon Infusion



8. CD62p

There was no significant difference when groups were compared after top-load with Oxygent or Hespan. Also, no significant difference was found within the groups when different time points were compared ($p>0.05$).



9. Alanine Aminotransferase Level

There was no significant difference when groups were compared after top-load at baseline, 0 and 3 hour point. However, after 24 hours, ALT had a trend to increase ($p=0.062$) in control group. Also, ALT was significantly increased at 24 hours and 4 days after top-load with Oxygent ($p<0.0003$) or Hespan ($p<0.0151$) compared with baseline.

Results (sheep behavioral monitoring)

All sheep subjected to blood sample analyses outlined above were observed behaviorally using non-interfering video camera to hard drive recording from 2 days prior to top-load procedure through the duration of the blood sample time points. Data is currently being analyzed and will be revealed in future reports.

Result summary: In the current study period, healthy sheep received intravenous infusion of Oxygent PFC, hestastarch, or saline/control. PFC animals showed no significant reduction of platelet count nor revealed significant activation of platelets when compared with control groups or compared with its baseline. More detailed data analysis is ongoing. Based on the original plan, each group needs 8 to 11 cases to reduce the data standard deviation.

Problems and solutions:

1. One PFC (Oxygent) has been tested and a second PFC used previously in blast TBI studies (PHER-O2) has not been delivered. **Solution:** The third PFC, "Perforan" was recently delivered and will be tested and compared with Oxygent.
2. Platelet functional assay: there were a few number of data drift away from baseline without clear reason. **Solution:** Sample values were doubled and repeatedly measured.
3. Sheep health is very important for the study. When there were sick sheep, it was difficult to distinguish ill sheep from PFC's side effect. We had lost 4 sheep in early summer time. **Solution:** Meeting with DAR veterinarians resulted in the vendor treating sheep with antibiotics before shipping and acclimation time post shipping was extended from 3 days to 7 days.
4. On November 25, 2013, laboratories suffered a big flood due to a street water pipeline that

was broken. Dirty water reached 18 inches in depth and remained for more than 48 hours. Sanger Hall, the building that housed the laboratories, had to be shut down for two weeks without power. All laboratories were evacuated and, experiments halted, and four sheep euthanized, which would have completed the phase I, top-load study. **Solution:** Laboratories were moved to another location in Sanger Hall. The laboratories are now up and running for the continuation of these studies. Insurance claims are underway for equipment damage (not essential to this study) and reimbursement of 4 sheep.



Key Research Accomplishments for year one:

1. Established the study which included protocol approval and development of large animal survival surgeries. 26 sheep were used for this reporting period.
2. A sheep hemorrhagic shock survival model for phase 2 of this study in 2014 was successfully developed. Both the sheep top-load and a hemorrhagic shock survival models passed VCU veterinarian observation.
3. Initial data analyses suggested that intravenous PFC infusion in healthy sheep did not result in thrombocytopenia or coagulopathy. These data are encouraging for FDA approval of further clinical trial study of PFC in the United States.

Reportable outcomes for year one:

1. Two first year medical students were awarded medical student summer research fellowships based on this study.

2. Based on model development and data from the current study, further grant applications are being planned.
3. The large animal survival hemorrhagic shock and trauma model has been established.
4. Manuscripts and abstracts are being developed based on the results of the current study.
5. Current study budget supports 3 full time employee and two part time employees.

Conclusion for year one:

1. Intravenous PFC infusion in healthy sheep did not significantly reduce platelet number. Functional assays did not show significant activation of platelet and white blood cells by either functional tests or by scanning EM.
2. These results suggest that further study of PFC is warranted as planned. This research project going forward is to assess whether the addition of PFC in the face of hemorrhagic shock increases the coagulopathy of shock and eventually in the combination of hemorrhagic shock plus blast traumatic brain injury.
3. Expanded experiments using PFCs other than Oxygent will continue for comparison and these data will be made available to the public (peer reviewed publications) as well as to manufacturer's of PFC so that approaches to the FDA for further clinical trials can be established.
4. At this time, independent activation of platelet's or white cells by Oxygent (our only tested PFC in this model for this reporting period) was not observed.

References N/A

Appendices:

Summary table of platelet bio-assay.

Parameter	Baseline		0 Hour		3 Hour		24 Hour		96 hour		7 Days	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
NATEM CT (sec)	685.71	129.17	483.71	45.62	572.57	84.03	674.14	128.72	669.29	123.86	730.43	111.24
	548.00	130.69	547.20	130.03	543.17	75.70	545.00	105.83	595.83	68.76	475.00	44.97
	848.67	146.61	769.17	84.91	853.00	47.00	905.83	125.43	1002.67	154.05	865.00	195.28
NATEM CFT (sec)	204.64	35.47	126.14	20.05	144.43	14.14	163.29	22.88	186.43	52.32	222.36	49.39
	137.17	31.96	187.20	49.83	149.17	28.67	141.67	28.06	160.83	18.31	122.60	6.36
	266.00	52.27	237.67	25.94	283.00	38.00	273.83	44.48	333.67	62.54	299.33	84.74
NATEM MCF (mm)	73.14	2.05	74.43	1.39	73.43	1.54	77.14	1.81	75.57	2.89	74.71	1.78
	77.50	1.96	75.70	2.75	76.83	1.92	77.67	1.43	74.17	1.01	75.60	1.91
	75.50	2.86	74.33	2.25	67.50	1.50	74.67	2.62	70.00	3.52	72.67	3.51
NATEM Angle (%)	57.57	4.52	68.00	2.98	65.64	2.37	63.43	3.18	61.71	5.88	57.71	5.22
	67.17	4.60	61.60	6.49	65.67	3.96	66.67	3.74	62.50	2.39	68.40	0.81
	52.83	6.56	54.17	3.32	46.00	3.00	52.50	3.20	45.67	6.33	51.33	8.32
INTEM CT(sec)	151.14	17.04	125.86	20.04	144.57	10.83	155.57	14.54	138.43	24.08	159.29	6.36
	142.00	17.91	151.80	18.88	149.67	13.87	147.17	17.75	143.67	4.30	139.20	9.31
	179.67	15.31	161.83	14.75	174.33	12.61	169.50	15.15	161.50	17.73	186.50	8.50
INTEM CFT (sec)	58.57	3.49	68.00	5.77	62.29	4.76	58.29	3.50	51.29	4.50	49.14	6.25
	52.00	1.71	67.40	8.35	64.67	10.69	60.83	4.20	69.17	4.30	64.60	5.23
	48.17	7.52	62.83	7.76	75.50	21.50	57.67	7.77	58.50	9.11	63.50	8.14
INTEM MCF (mm)	76.00	1.45	75.86	0.94	76.29	1.27	78.57	0.95	78.86	1.14	78.57	1.88
	78.67	2.22	79.60	2.93	78.50	1.82	80.17	1.82	76.67	1.84	75.40	1.86
	80.17	2.07	80.00	2.48	75.00	2.00	79.83	2.30	78.00	2.02	77.00	1.86
INTEM Angle (%)	78.86	0.46	76.57	1.07	78.14	0.74	78.57	0.84	79.86	0.96	80.00	1.20
	74.50	5.31	76.80	1.69	77.33	2.12	78.00	0.82	76.50	0.76	77.40	0.98
	80.17	1.47	78.67	1.28	76.00	3.00	78.50	1.65	79.00	1.51	77.67	1.61
EXTEM CT (sec)	74.29	18.32	75.29	19.75	123.57	48.86	55.29	5.80	64.57	5.58	78.29	9.45
	51.67	4.90	61.40	9.66	75.00	12.70	56.17	7.05	61.83	9.02	62.20	5.30
	78.00	4.90	78.00	6.42	67.50	9.50	75.00	2.94	74.17	7.23	75.33	7.00
EXTEM CFT (sec)	84.29	9.85	105.14	7.65	102.57	9.60	84.43	8.04	72.14	7.28	73.71	9.75
	88.17	15.23	85.60	22.42	109.83	10.25	86.83	10.09	92.67	7.92	97.20	4.90
	66.83	6.52	82.17	16.02	119.00	22.00	79.67	8.79	77.67	12.33	93.17	6.60
EXTEM MCF (sec)	74.86	2.90	77.00	1.09	77.57	1.17	78.86	1.30	77.86	1.06	79.86	1.30
	78.67	2.50	79.80	2.65	80.00	1.71	79.50	1.45	75.00	2.35	74.80	2.01

	82.17	1.56	80.50	2.14	75.50	1.50	80.17	2.24	78.33	2.06	77.33	1.94
EXTEM Angle	76.71	1.95	74.14	1.14	74.71	1.57	78.86	1.08	78.43	1.07	77.71	1.87
	78.33	1.58	70.80	3.40	72.50	2.43	75.67	1.84	75.83	1.25	73.20	1.77
	78.83	0.79	78.50	2.40	72.50	0.50	77.67	1.69	79.17	1.30	76.83	0.95
Platelet Count	515.91	65.13	426.53	120.02	443.66	44.28	457.85	50.12	401.29	46.23	438.69	58.63
	605.31	41.09	438.72	59.23	527.69	41.66	488.31	47.09	434.17	45.51	432.05	76.77
	493.03	71.68	468.03	97.83	415.33	49.67	473.72	107.89	423.66	119.34	432.16	94.83
Fibrinogen	222.38	26.30	213.75	29.90	215.00	26.34	270.38	35.01	351.38	93.87	267.71	61.58
	242.67	49.02	223.17	48.99	245.67	40.06	255.83	47.61	224.83	33.22	212.40	52.12
	234.83	31.46	264.50	50.95	157.50	3.50	241.67	35.87	181.00	17.46	165.00	5.82
Coll/Epi PFA	264.14	23.66	213.43	21.38	222.33	35.29	186.29	30.68	249.00	33.95	256.29	29.18
	300.00	0.00	278.83	21.17	259.83	25.61	233.17	30.32	270.80	19.95	300.00	0.00
	153.33	10.09	177.50	28.40	198.50	101.50	244.33	35.39	283.33	16.67	235.17	30.63
Coll/ADP PFA	110.71	32.47	125.57	30.20	149.00	48.38	104.29	16.57	160.17	44.34	128.71	31.40
	188.83	49.74	81.50	8.53	111.33	37.97	161.83	41.56	85.20	10.34	82.25	12.98
	75.50	8.80	122.17	36.15	102.50	34.50	82.33	10.54	178.33	39.13	123.67	36.40
FOT (min)	9.43	2.45	8.14	0.94	9.43	2.27	10.00	1.99	7.86	2.59	12.14	2.64
	10.00	2.62	8.67	2.68	9.17	2.27	8.33	2.70	13.33	2.17	8.00	1.45
	16.00	1.63	13.33	2.36	15.00	5.00	16.33	1.82	16.83	2.01	11.00	3.00
CEM (kdynes/cm2)	26.46	9.59	20.65	2.82	18.79	5.41	24.15	11.80	39.56	13.01	21.88	8.25
	32.48	15.98	50.11	18.24	38.06	16.41	49.33	17.70	13.83	5.43	32.91	6.17
	8.75	5.19	18.81	10.59	15.43	15.43	10.19	4.12	6.10	3.88	23.69	12.79
PCF (kdynes)	7.72	3.14	7.06	1.03	7.15	2.68	6.49	2.66	11.88	3.39	5.55	2.24
	9.70	4.14	14.10	4.77	10.52	3.62	12.09	4.24	3.45	1.31	8.49	1.70
	2.98	1.99	4.51	2.55	2.80	2.80	1.75	0.69	1.65	1.05	7.33	3.98
Liver Enzyme (ALT)	15.14	0.88	15.71	0.87	21.14	2.89	35.14	5.51	32.14	4.63	20.43	2.35
	15.33	1.12	14.17	1.89	17.50	2.49	23.00	2.17	27.40	4.23	20.20	3.65
	12.83	1.87	12.50	1.61	15.50	5.50	20.00	4.06	18.83	3.57	18.17	2.15
Platelets CD62p	16.00	3.71	12.34	3.21	10.47	1.91	14.61	3.99	18.97	4.42	12.05	0.66
	11.59	2.03	17.37	7.62	11.64	1.84	9.18	0.84	13.32	2.23	12.50	1.00
	12.53	2.32	16.47	4.13	9.32	2.10	17.20	7.76	11.63	3.09	8.05	1.15
vWF: Ag	11.47	2.79	10.94	2.68	10.79	2.48	10.83	2.42	13.43	0.78	11.01	2.55
	5.40	1.30	4.64	0.97	5.01	0.83	4.39	0.74	4.67	0.72	4.65	0.72
	13.97	2.72	13.31	2.77			12.76	2.53	12.81	2.78	12.93	2.77
Collagen Agg (Ohms)	11.00	1.12	11.71	1.22	11.36	1.67	12.50	1.74	11.07	1.46	12.36	2.57
	13.08	1.57	12.17	0.71	10.42	1.36	9.25	2.09	12.42	1.38	10.60	1.37
	12.92	0.80	10.67	1.84	7.50	1.50	10.00	1.16	11.17	2.19	10.58	1.17

ADP Agg (Ohms)	10.64	0.57	10.64	0.99	10.00	1.13	10.29	1.27	10.07	1.18	10.79	1.34
	10.83	0.76	10.58	0.82	9.58	1.00	8.92	1.98	9.83	0.92	11.00	1.05
	13.17	1.05	36.17	25.47	8.00	2.00	8.33	1.17	9.83	1.35	9.42	1.21
CAT lag	4.84	0.69	5.50	0.87	4.37	0.50	5.44	0.68	4.27	0.70	5.35	1.33
	4.79	0.56	4.70	0.74	4.33	0.79	4.33	0.81	3.75	0.32	4.64	0.31
	5.87	0.62	4.90	0.67	6.96	0.05	4.73	0.28	4.35	0.25	4.61	0.49
CAT ETP	854.88	147.53	721.72	129.09	726.76	173.81	723.14	147.49	731.70	176.56	763.57	99.58
	872.10	122.88	890.62	117.35	820.09	67.25	817.59	96.81	554.15	91.29	631.76	96.32
	981.66	167.45	860.80	220.39	1136.07	399.78	848.69	80.53	769.04	122.79	700.00	121.75
CAT Peak	47.17	9.36	39.44	11.45	47.10	13.38	44.53	12.92	52.07	17.36	47.43	6.84
	54.59	13.61	52.26	12.59	54.05	8.27	50.41	10.05	31.75	5.95	34.87	4.61
	59.58	17.18	46.50	10.31	60.73	12.75	43.06	6.41	45.05	12.74	36.15	9.68
CAT Tip	16.78	1.32	17.69	1.80	14.47	1.31	16.01	0.92	15.74	2.28	16.69	1.50
	18.69	2.18	18.82	2.42	15.51	1.44	15.51	1.90	15.79	1.92	16.69	0.83
	21.36	1.25	17.78	1.51	21.04	3.79	19.54	1.54	18.32	1.90	20.73	2.28
Neutrophils	138.40	6.86	141.33	26.80	126.40	25.38	182.25	36.46	136.00	32.33	136.00	34.39
	184.80	38.91	138.40	21.51	180.75	22.67	166.33	41.35	145.67	3.53	207.00	
	134.33	16.38	119.17	15.19	84.50	32.50	145.80	8.21	158.00	26.13	96.67	22.73
Monocytes	147.20	20.15	188.67	38.87	159.20	23.98	178.50	36.97	181.00	49.00	199.75	42.86
	202.40	29.28	194.00	25.87	227.75	38.62	249.33	93.20	239.00	1.00	291.00	
	142.50	20.23	147.17	16.04	111.00	7.00	157.00	33.19	165.25	12.66	105.67	22.10

Panel color code explanation

Parameter	147.20	Oxygent group n=7
	202.40	Hespan group n=6
	142.50	Control group n=6